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Reciprocal Recombination in Prophage Lambda [1]

MATTHEW MESELSON
Biological Laboratories, Harvard University, Cambridge, Massachusetts

ABSTRACT Well-separated prophage markers in an *E. coli* λ/F′ (λ) partial diploid recombine reciprocally.

Although bacteriophages and bacteria have proven to be uniquely suitable forms for direct structural and biochemical investigations of the molecular basis of genetic recombination, there is one important aspect of recombination which has so far been examined almost exclusively in eukaryotes. This aspect is the reciprocity of recombination. The experiment reported here represents the first part of a study of recombination in prophage lambda that is aimed at determining the detailed patterns of reciprocity or nonreciprocity associated with various modes of genetic exchange in both wild-type and recombination- or repair-deficient bacterial strains. It is hoped that the information obtained can be correlated with structural and biochemical findings in phage lambda and *Escherichia coli* to help provide an understanding of how recombinant DNA molecules are formed.

This paper describes some features of recombination and segregation in *E. coli* partial diploids carrying a lambda prophage on the bacterial chromosome and a differently marked lambda prophage on an F′ episome. The investigation of recombination and segregation in this system is similar to mitotic half-tetrad analysis in diploid eukaryotes. It is found that recombination of well-separated lambda markers is very often reciprocal. Also, certain other features of recombination and segregation are formally similar to those found in diploid eukaryotes.

PLAN OF THE EXPERIMENT

Bacterial strains heterozygous for prophage lambda

The occurrence of F′ episomes (Jacob and Adelberg, '59) makes possible the construction of *E. coli* strains heterozygous for part of the bacterial genome. An F′ episome may be regarded as a small accessory chromosome, part of which is homologous to a segment of the bacterial chromosome. Several F's are known, each possessing homology with a different region of the bacterial chromosome. An F′ isolated by [E. L.] Wollman carries the insertion site for prophage lambda and may therefore be used to construct strains heterozygous for lambda markers. For the present study, we constructed a strain which will be called the "parental strain" and which has a relevant genetic constitution that may be represented as

F′	+	+	+
Chromosome	*c*	*mi*	*h*

Although the strain is rather stable genetically, recombination and segregation do occasionally occur in the course of bacterial growth and were studied by determining the types of prophages present in a large number of unselected individual cells, each independently and remotely descended from the parental strain.

Determination of prophage content

The lambda markers employed are without significant effect on the properties of the lysogenic strains carrying them. Each of the markers — *c* (a c_{II} clear), *mi* (minute), and *h* (host range) — may easily be scored by its effect on the morphology of plaques formed by free phages. Free phages derived from both the chromosome and the F′ episome are spontaneously liberated at a low frequency during the growth of lysogenic bacteria. Thus, the types of prophages present in a given cell will be the same as those which predomi-

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nate among the free phages found in a culture grown from that cell, barring the rare occurrence of a recombination in the earliest divisions. In other words, the prophage content of a single cell may be determined simply by allowing the cell to produce a culture, plating the associated free phages by standard techniques, and observing the plaque types which predominate. This method for ascertaining prophage content is rather like the scoring of spontaneously occurring haploids to determine the genetic constitution of a diploid eukaryote.

RESULTS

The method outlined above was used to determine the prophage content of 5735 unselected cells, each of which had essentially descended independently through an average of approximately 30 generations from the parental strain. The results are presented in table 1. Detailed procedures and the results of certain control experiments will be published elsewhere.

The parental strain is diploid for prophage lambda

It may be seen in table 1 that the large majority of cells tested (about 90%) contain the same two prophage types contained by the parental strain. Another 8%, or 437, contain a pair of prophage types, at least one of which is recombinant. No cells were found to contain more than two different prophage types. We conclude from this fact that the parental strain is truly diploid for prophage lambda, in the sense that the unit of prophage inheritance consists of just two nonsister prophages. Tests to be reported elsewhere indicate that the 162 cells with only one prophage type are homozygous diploids rather than haploids.

Recombination frequencies

By giving single weight to heterozygous diploids and double weight to homozygous diploids, the frequency of each prophage type may be found directly from the frequencies of the various diploids that contain it. The frequency of each of the eight possible prophage types is given in table 2, and it can be seen here that each type occurs with an overall frequency nearly equal to that of its reciprocal.

Upon lysogenization the lambda chromosome undergoes a specific rearrangement that is reversed upon induction (Calef and Licciardello, '60; Campbell, '62). The prophage map order *c–mi*–R–L–*h* is a permutation of the vegetative map order L–*h*–*c*–*mi*–R, where L and R designate the "left" and "right" ends of the vegetative map. The order *c–mi–h* inherent in the recombination frequencies of table 2 is that of prophage lambda, as expected.

The interval *c–mi* is found to be slightly shorter than the interval *mi–h*. This is to be expected if previously measured vegetative map lengths are proportional to lengths found in prophage crosses of the sort that we are dealing with here, and if the frequency of prophage recombination in the interval L–R is negligible.

Reciprocal recombination

We now come to that aspect of our results which we wish to emphasize most:

TABLE 1

Classification of 5735 unselected cells descended from the diploid strain c mi h/F′ + + + *

+ + +	*c mi h*	*c* + +	+ *mi h*	*c mi* +	+ + *h*	*c* + *h*	+ *mi* +	
94	*5136*	45	30	52	11	17	3	+ + +
	58	27	28	11	54	3	11	*c mi h*
		2	36	4	8	3	1	*c* + +
			2	8	0	2	1	+ *mi h*
				1	68	0	5	*c mi* +
					4	1	0	+ + *h*
						0	8	*c* + *h*
							1	+ *mi* +

* The value in each position is the number of occurrences of cells containing prophages of the type designated at the end of the corresponding row and column. Diploid types heterozygous at all loci are in italics. Diploids with two different prophages can occur in two configurations, depending on which prophage is in the F′ episome and on which is in the bacterial chromosome. The table does not distinguish these different configurations.

TABLE 2
Prophage recombination frequencies

Recombinant	Number	Frequency
		%
+ *mi h*	109	0.95
c + +	129	1.12
+ + *h*	150	1.30
c mi +	150	1.30
+ *mi* +	31	0.27
c + *h*	34	0.30

Interval	Number	Frequency
		%
c–*mi*	303	2.64
mi–*h*	365	3.17
c–*h*	538	4.67

the recombination of *c*, *mi*, and *h* is often genuinely reciprocal.

It may be seen in table 1 that many nonparental diploids contain a pair of reciprocally related prophage types and that many do not. However, these observations cannot be taken in themselves as evidence for or against reciprocal recombination. On the one hand, for example, nonreciprocal diploids would arise if recombination occurs reciprocally but in a multistrand group, as in higher organisms, so that reciprocally related prophages might segregate to different daughter cells after recombination. On the other hand, the frequent occurrence of reciprocal diploids does not necessarily mean that recombination is geniuinely reciprocal. Instead, if some cells undergo a much greater number of recombination events than do other cells, it might often happen that a given recombinant and its reciprocal would be formed in the same cell quite independently. In that case, the presence of a recombinant would be positively correlated with the presence of its reciprocal in the same cell, but the cause of the correlation would be intercellular heterogeneity of the intensity of recombination rather than true reciprocity.

In order to demonstrate reciprocity, let us consider recombination in the intervals *c*–*mi*– and *mi*–*h*. We have seen that the two intervals are nearly equal. Thus, if there is no true reciprocity of recombination in these intervals, we would expect the prophage *c* + +, for example, to be found as often with + + *h* as with its reciprocal, + *mi h*. More generally, the nonreciprocal diploids, *c* + +/+ + *h* and + *mi h*/*c mi* +, should occur as often as the reciprocal diploids, *c* + +/+ *mi h* and *c mi* +/+ + *h*. Yet this was not observed. Instead, we found that the former class comprises only 16 diploids, whereas the latter includes 104 (see table 1). This result proves that recombination in the intervals *c*–*mi* and *mi*–*h* is quite often genuinely reciprocal.

Homozygosis

If *c*, *mi*, and *h* recombine reciprocally, how can we account for the frequent occurrence of diploids homozygous at one or more loci? Nothing in our results rules out the possibility that recombination is only sometimes reciprocal and that homozygosity results from nonreciprocal recombination. However, homozygosity can be explained without invoking nonreciprocal recombination if the products of a given reciprocal exchange sometimes fail to remain together during subsequent cell divisions. By analogy with higher forms, this might happen if recombination occurs at a multistrand stage and if partners to a given reciprocal exchange do not necessarily segregate together in the formation of daughter cells. A detailed consideration of this possibility would lead us rather far from the consideration of reciprocity, the main subject of this paper. It will suffice to say that the distribution of frequencies of the diploid cell types found in the present experiment agrees fairly well with that calculated on the basis of postulates derived from the study of reciprocal mitotic recombination in eukaryocytes. A detailed account will be published elsewhere.

DISCUSSION

The present experiment shows conclusively that recombination of relatively well-spaced lambda prophage markers is very often reciprocal. Similar but less decisive evidence for reciprocal recombination of the *E. coli* chromosome with an F′ episome has been obtained by Herman ('65), who employed two markers in the *lac* operon. Proof of the possibility of reciprocal recombination in bacteria adds support to Campbell's ('62) appealing hypothesis for the chromosomal insertion and excision of bacterial episomes by means of reciprocal

recombination in a region of lambda that is homologous to the lambda insertion region of *E. coli*.

The chromosome of free phage lambda consists of a single DNA duplex. The prophage lambda chromosome is almost certainly the same duplex inserted into the linear continuity of the DNA duplex that comprises the chromosome of *E. coli*. Physical and genetic studies of *vegetative* lambda recombination have shown that duplex phage chromosomes break and join, giving recombinant molecules in which segments of different parentage are connected covalently via regions of hybrid DNA (see Meselson, '67). If the reciprocity of prophage recombination is also characteristic of vegetative lambda recombination, we may conclude that structures of this sort are formed in reciprocal pairs. A number of somewhat indirect arguments incline us to accept this view, although attempts to detect reciprocal recombination by genetic analysis of single bursts in vegetative lambda crosses and in other vegetative phage crosses have, on the whole, been unsuccessful (Hershey and Rotman, '49; Jacob and Wollman, '54; Bresch, '55). However, the probability of recovering reciprocal recombinant types in a given single burst could be so greatly reduced by losses inherent in phage replication and maturation that reciprocity would be largely obscured.

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OPEN DISCUSSION

CURTISS [1]: I would like for you to clarify something for me, and maybe for others. As we all know, the bacterial chromosome is circular, and it's widely believed that F′ elements are circular. Therefore, as shown by a number of workers, one reciprocal exchange between an F′ element and a chromosome will initially result in a tandem duplication which will behave as an Hfr donor. A second reciprocal exchange will then be necessary to regenerate the F′ factor. So how do these events affect your calculations? Also, the length of the circularized F′ could be important in terms of your homozygosity data, since the lambda markers could be close to F.

MESELSON: The genetic map length of the interval between *o* and *h* may be only a small fraction of the complete map of the F′ element. If this is so, then the effects of circularity, if present, should be relatively weak. In this connection, Dr. Kenichi Matsubara has found that the F′ element I have used sediments as though it is many times larger than the lambda chromosome.

THOMAS [2]: Could you go over any assumptions that you have in regard to whether spontaneous lysis is an adequate assay for the prophages, regardless of the structures in which they happen to reside?

MESELSON: When a culture is saturated, there are about 10^7 free phages per ml. When there are two or more phage types in a pure culture, just two of the types are found to constitute a majority of the phages. These two are present in roughly equal numbers. If the bacterial strain is allowed to conjugate with a lambda sensitive female, only one of the majority phage types is transferred. I interpret these observations to mean that the two majority phage types produced by a given culture are the prophage types it contains and that the transferable type is on the F, while the other is on the chromsome.

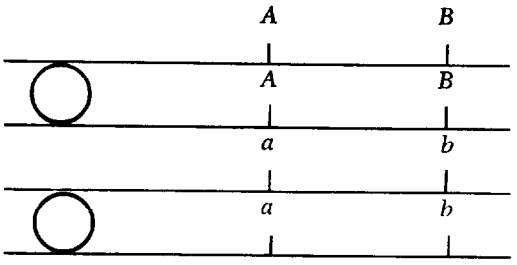
CHMN. BAKER [3]: At this time I would like to call on Dr. Herschel Roman, who has some remarks to make.

ROMAN [4]: After hearing about the clever tactics used by Dr. Meselson to study recombination in the bacterial system, I have come to feel that yeast may offer certain advantages in recombination studies of the kind that he has made. Yeast is useful in the context of this discussion because it has a stable diplophase in which recombination occurs, and because all of the viable products of recombination can be recovered.

The results that I want to describe are derived from diploid cells in which a pair of homologous chromosomes is marked as

[1] Roy Curtiss III, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
[2] Charles A. Thomas, Jr., Harvard Medical School, Boston, Massachusetts.
[3] William K. Baker, University of Chicago, Chicago, Illinois.
[4] Herschel L. Roman, University of Washington, Seattle, Washington.

shown schematically in the following diagram:



This diploid divides mitotically. The centromeres are distributed as in mitosis, and the daughter cells normally reproduce the genotype of the mother cell. The four-strand stage is shown because the cells at the start of our experiments can be shown to have already replicated their DNA for the division that follows. We are looking in this system for evidence of the two types of recombination that Dr. Meselson has referred to: conversion and crossing-over. Conversion is generally characterized by nonreciprocal recombination; crossing-over is characterized by reciprocal recombination. Homozygosity for the recessive allele *a* in sectored colonies signals the occurrence of recombination, either at the *A* locus or in the region from *A* to the centromere. Are there conditions under which nonreciprocal and reciprocal recombinations are separable? Our observations are summarized in [discussion] table 1.

In this table reciprocal recombination is identified by the recovery of two types of cells, homozygous *AA* and homozygous *aa*, both of which arise from the same recombinational event. Nonreciprocal recombination is also identified by its products. In this case, however, one of the two types is *Aa* and the other is *aa*. The complementary nonreciprocal products we would also expect to recover, *AA* and *Aa*, are not detected by our techniques. Spontaneous recombination between the centromere and *A* occurs with a frequency of about 10^{-4}. We have not characterized the products.

When ultraviolet [UV] light is used as the inducing agent, 75% of the recombinational events are reciprocal. With UV doses that are nonkilling a total recombination frequency of about 1% is obtained.

An interesting recent observation that Susan Effs and I have made is that chemical mutagens — specifically, EMS, nitrosoquanidine, and hydroxylamine — yield mainly nonreciprocal recombination. With EMS, more than 95% of the recombinational events that are recovered are of this type. The frequency of recombination depends on the amount of treatment. With 75% survival, the frequency is approximately 5%. With 35% survival, it is approximately 10%. These extraordinarily high frequencies are not the property of the *A* locus alone, however, for three other loci have been tested and all have responded to give high levels of recombination.

Reciprocally recombinant cells are recovered with a frequency of 1 to 1. Half-sectored colonies are obtained on the average. There is a spectrum of sector size, but this spectrum is identical to the spectrum that is obtained when one moves *aa* and *AA* cells next to each other in pairs, and with a micromanipulator the two cells develop into the colony. Reciprocal recombinations therefore segregate in the first division following treatment.

The sector size, in the case of nonreciprocal recombination, is different from the

DISCUSSION TABLE 1

Frequencies of spontaneous and induced reciprocal and nonreciprocal recombination

Treatment	Relative frequencies of:		Frequency of total recombination	Survival
	Reciprocal recombination *	Nonreciprocal recombination †		
	%	%		%
Spontaneous	—	—	10^{-4}	100
UV	> 75	< 25	10^{-2}	95–100
EMS	< 5	> 95	5×10^{-2}	70

* Reciprocal recombination types: *AB/AB* and *ab/ab*.
† Nonreciprocal recombination types: *AB/ab* and *aB/ab*.

pattern for reciprocal recombination. We do not have enough data to be precise about the difference, but it is a fair guess that the sector size will be close to ¼ *aa*, as would be expected if segregation of the products is delayed until the second division of the treated cell.

The reciprocal recombinants are, in the main, not limited to *AA* or *aa*, for the distal markers on the same chromosome also become homozygous, in the relation that would be expected from the reciprocal breakage-reunion of duplexes in the centromere to *A* region. This relationship is illustrated in the schematic diagram. For the nonreciprocal events, however, the distal markers remain in a heterozygous condition.

It is tempting to compare these results with those of Dr. Meselson and to say that reciprocal recombination is the consequence of breakage and reunion of DNA duplexes in homologous chromosomes at the four-strand stage. Nonreciprocal recombination could then be thought of as being the result of the formation of hybrid DNA, a kind of interchromosomal transformation that sorts out in the second division.

MESELSON: How close is *A* to the centromere?

ROMAN: *A* is about 35 meiotic crossover units from the centromere, and therefore not very close. But mitotically it is very close, since the spontaneous recombination frequency is about 10^{-4}.

MESELSON: Then would it be correct to conclude that recombination is extremely intense all along the chromosome?

ROMAN: Exactly.

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